STUDIES ON ALLELOPATHIC BEHAVIOUR OF CERTAIN WEEDS IN RELATION TO POTENTIAL FOR WEED MANAGEMENT

DISSERTATION

Submitted in Partial Fulfilment of the Requirements
for the Award of the Degree of

Master of Philosophy

IN

BOTANY

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ALIGARH MUSLIM UNIVERSITY
ALIGARH (INDIA)

1994

D E D I C A T E D

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Dated. July 13,1994

CERTIFICATE

This is to certify that the dissertation entitled **"Studies on Allelopathic behaviour of certain weeds in relation to potential for weed management"**, submitted to the Aligarh Muslim University, Aligarh, in partial fulfilment of the requirements for the award of the degree of **Master of Philosophy**, is a bonafide work carried out by **Miss Asma Parveen.** No part of the dissertation has been published or submitted for any other degree or diploma.

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ACKNOWLEDGEMENT

In the name of Allah the most beneficient,

the most merciful.

I feel greatly honoured in expressing my humble thanks and gratitude to **PROF.WAZAHAT HUSAIN,** Chairman, Department of Botany, Aligarh Muslim University, Aligarh,for the support and able guidance that he bestowed on me due, to which this piece of work has materialized.

I would also like to extend my sincere thanks to all members of the Ecophysiology Unit Punjab University, Chandigarh, in particular to **DR. R.K.KOHLI** and **MISS DAIZY BATISH.** Their suggestions and help proved to be greatly valuable to me.

I am heavily indebted to all my friends, REEMI, TALAT, LUBNA, RIZWANA, ANEESA and SABA for their kind co-operation.

Mr. SUHAIL AKHTAR NAQVI deserves a special mention for his help and encouragement.

In the last but not the least I would like to thank my most loving and caring parents who have always been the light of my life.

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C O N T E N T S

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I N T R O D U C T I O N

According to Jethro Tull (1731), weeds are defined as **"A weed is a plant growing where it is not desired."** This definition of a weed however is quite arbitrary as some plants would be quite undesirable at one place or time and desirable at another.

Cynodon dactylon (Linn.) Pers. or bermuda-grass for instance is a highly troublesome weed in crop fields, but in villages it is used as a fodder. Opuntia [Tourn.] ex Miller prickly pear growing in deserts is not a weed but in many countries including India it has invaded even fertile lands.

Hence a better definition of a weed would be that weeds are plants growing in places and at times when we wanted either some other plants to grow or no plants to grow at all.

Weeds though unwanted plants are important for the sustenance of natural climax eco-system. However, they assume harmful proportions when they compete with the crop systems for light, minerals and space in agricultural and

horticultural sectors, thereby causing economic losses. Directly they reduce the crop yields and indirectly they elevate farm production costs. Besides, their negative values are depicted during harvesting, marketing, storage and dockage of weedy crops.

WEED MENACE

Weeds are ubiquitous and their effects create enormous losses. The foremost damage by weeds is on farm as detriment to cropping system. In India the annual loss in crops yields by weeds is estimated to be 10%. Directly they reduce the crop yields and indirectly they elevate farm production costs by energy spent in controlling them.

Weeds may mar the quality of farm produce in many ways. Contamination of food grains with seeds of weed like Lolium Linn. Agrostemma Linn. Senecio [Tourn] Linn. and Allium spp. [Tourn] Linn. are often responsible for serious consequences to the consumers.

Lantana camara Linn. induces hyper-sensitivity to animals feeding upon it. Rhododendron Linn. has been found responsible for diaerrhoea in milching animals. Sorghum halepense (Linn. Pers.) is poisonous to grazing animals. Thorny weeds cause sores in the hooves of animals.

Health, comfort and work efficiency of man are adversely affected by weeds. Pollens of Ambrosia Linn, and Franseria Cav. cause hay fever and asthma. Dermatitis is another common allergy resulting from direct contact with weeds like Parthenium L., Rhus [Tourn] L. and Helenium L., spp.

The hair of Urtica spp.[Tourn]L. "stinging nettle" cause severe itching and inflammation.

In addition to the above mentioned harmful effects weeds also contaminate water-bodies, cause damage to industry and public utilities and loss of forest and wood-land products. In addition they also cause a deterioration of aesthetics by making the scenery dull.

WEED CONTROL

In weed control we seek to limit the unwanted growth of plants, both-in-space and time, without any attempt to eliminate them from the scene, for their complete removal can result in ecological imbalance. Weed control can be either mechanical, chemical or biological. Mechanical control can be practised in small areas and for limited infestations. Chemical control with weedicides and herbicides is currently practised all over the world for

dense infestations. However their indiscriminate use has led to an ecological imbalance, pollution of soil and air and a serious threat to human health.

Biological weed control though seems fascinating, however, it is a comparatively neglected area of weed control. But fortunately for us it is on the top of the priority list of weed scientists, agriculturists and agronomists. It is in this regard that a new approach dealing with plants Vs. plants is gaining momentum and it is here that allelopathy comes into play.

ALLELOPATHY

Plants produce many metabolites that have no known utility in plant growth and development. Thus, the concept that plants produce chemicals toxic to themselves and to other plants and differ in their response to these chemicals is not illogical.

Based on their known chemistry, such chemicals can be expected to be harmful if present in sufficient concentration and proximity to a neighbouring seed or growing plant.

Molisch (1937) coined the term allelopathy to refer to bio-chemical interactions between all types of plants

including micro-organisms. This definition as given by Molisch covers both detrimental and beneficial reciprocal bio-chemical interactions.

Rice (1974) has defined allelopathy as any direct or indirect harmful effect by one plant (including micro-organisms) on another through production of chemical compounds that escape into the environment.

A very important point concerning allelopathy is that it's effect depends on a chemical compound being added to the environment. It is thus separated from competition which involves the removal or reduction of some factor from the environment that is required by some other sharing the habitat. Factors that may be reduced include water, minerals, food and light.

HISTORICAL BACKGROUND

Rice (1974) traces the beginnings of allelopathy to De Candolle in the early 1800's who was among the first to suggest that some plants excrete substances injurious to other plants. This suggestion was based on the observation that some crop plants grow poorly in association with certain weeds or other crops and in soil following other crops.

It was nearly 50 years before the possibility again emerged with a report by Stickney and Hay in 1881 of the harmful effects of walnut trees on the growth of other plants beneath them.

Massey (1925) indicated that a deleterious root relationship exists between walnut and tomato plants. Keever (1950) observed that horse-weed (Conyza canadensis, Linn.) disappeared quickly from abandoned yields.

ALLELO-CHEMICALS

Chemicals that impose an allelopathic influence are termed as allelo-chemicals or allelo-chemics. Allelo-chemicals may be produced in any or all the parts of the plants such as roots, seeds, leaves etc.

According to Rice (1974, 79) the quantity of allelo-chemicals produced increases in UV light and in long day photo-periods. Quantities are also greater in conditions of mineral deficiency and under drought stress. Application of plant growth regulators, such as 2, 4-D and Maleic hydrazide and of other allelo-chemicals stimulates the production of allelo-chemicals.

Allelo-chemicals can enter the environment in four ways, i.e. either by

1) Volatilization
2) Leaching
3) Exudation, and
4) Decomposition,

below is a short description of each method as given by Putnam in 1985.

1. **VOLATILIZATION:** Living plants may emit volatiles which are inhibitory to their neighbours. Species within the genera Artemesia Linn., Eucalyptus L. and Salvia L., are believed to impose their allelopathic influences at least in part through the release of volatiles.

2. LEACHING: A variety of chemicals may also be leached from plant leaves or stems by rain-water. Recent work by Putnam (1985) indicates that velvet-leaf (Abutilon theophrosti Medic.) excudes phyto-toxins through it's trichomes (hairs on the stems and petioles). The inhibitors are easily removed by misting with water.

3. EXUDATION: It is another potential method of toxin release by roots. By the use of circulated nutrient solution or chemical trapping on adsorptive columns, a number of allelo-chemicals have been detected. Recently some 16 benzoic, cinnamic and phenolic acid compounds were

trapped from nutrient solutions in which bigalta limpo-grass (Hemarthia altissima Hubbard.) was grown.

4. **DECOMPOSITION:** Dead or decaying plant material may provide a major source of allelo-chemicals. Some of the best known of these are the cyanide - containing glycosides, which are degraded to produce toxins like cyanide and benzaldehydes. In addition a number of phenolic compounds are released by the decomposing plant residues. under certain soil conditions, it appears that they may exert toxic effects. According to Alan R. Putnam (1980) the management of cover crops and surface residues can greatly reduce germination and growth of several annual weed species.

POTENTIAL OF ALLELOPATHY FOR WEED MANAGEMENT

A tremendous amount of research has been conducted during the past century designed to elucidate the allelopathic responses involved in plant / plant interactions. Essentially all of such research has been concerned with explaining the observed phenomena of allelopathic responses. There have been few studies directed towards the use of allelopathy as a practical means of directly controlling weeds.

Putnam and Duke (1974) hypothesized that many presently cultivated species may have possessed allelopathic substances when growing in their wild habitat. Such a trait could have been lost through domestication with intensive breeding and selection for specific desirable characteristics. To evaluate their hypothesis they obtained 526 accessions of Cucumis sativus Linn. and 12 accessions of eight related Cucumis species representing 41 nations of origin. Each of the accessions were grown with two indicator weed species. There were substantial differences in the ability of the accessions to influence weed growth. Growth of Brassica hirta (Moench) ranged from 4 to 125 per cent of growth of the controls with a mean of 78 per cent.

The range for Pancium milliaceum L. was 7 to 126 per cent with a mean of 66 per cent of growth of controls. This assumes that allelopathy can contribute to the competitive ability of crop species against important weed species under field conditions.

According to Alteri and Doll (1973) the practical use of allelopathy is complex since these interactions rarely occur so markedly under normal field conditions as in the glass-house. It is probable that most of the toxins are secondary chemical compounds stored in the foliage tissues and rarely released into the environment in effective quantities. Before attempts are made to control weeds by allelopathic means the following points should be considered.

1) Intensification of programmes to identify allelopathic interactions between crops and crops, crops and weeds, weeds and weeds etc.

2) Identification of the parts of the plants which contain inhibitors and of the ways in which the inhibitors are released in to the environment.

3) Investigation of the effects of residues from plants of different ages and from different plant parts.

4) Chemical identification of the inhibitors and their mechanisms of action.

The present study aims at using allelopathy as a tool to work with weeds against weeds. Our approach is to isolate compounds from weeds having the ability to suppress other weeds. These compounds can serve as natural weedicides and herbicides. According to Rizvi and Rizvi (1984) the use of allelo-chemicals as natural herbiciders would be advantageous over synthetic ones because these:

- may be easily metabolized.
- may not be toxic to crop plants.
- may provide a means to decrease fossil fuel energy consumption in crop protection.
- may be incorporated by genetic manipulation in the cultivators, to provide an inexpensibe, safer and permanent means means of biological control.

Along with the above mentioned benefits such compounds are eco-friendly and pollution-free.

In highly developed countries such chemicals released through such a phenomenon are being marketed as natural herbicides. In India however very little work has been done in this regard.

Some plants such as Ecalyptus globulus Labill., Helianthus annus L., Parthenium hysterophorus L., Ageratum conyzoides Linn. Chenopodium rubrum Forsk., Chenopodium ambroisoides L. and Anisomelis ovata R.BNr are very promising plants for the extraction of such allelo-chemics and their possible use as herbicides.

REVIEW OF LITERATURE

Allelopathy in nature plays many roles in eco-system management in agriculture and forestry. The relevant literature chosen for the present study can be studied under the following headings:

1) Allelopathic effect of weeds on crop plants.
2) Allelopathic effect of crops on weeds.
3) Allelopathic effect of weeds on weeds.
4) Mechanism of action of allelo-chemicals.
5) Allelopathy and weed management.

1) ALLELOPATHIC EFFECTS OF WEEDS ON CROP PLANTS:

De Candolle (1832) suggested that the soil sickness problem in agriculture might be due to the exudates of crop plants and that rotation of crops could help alleviate the problem.

Overland (1966) pointed out that "smother crops" were often planted in the past to suppress the growth of weeds. Some of these included barley, Hordeum vulgare L., rye Secale cereale L., Sorghum vulgare Pers; buck wheat, Fagopyrum esculentum Moench; sweet clover Meliotus Juss and sunflower Helianthus annus. L.

She demonstrated that root exudates of barley caused inhibition of germination and growth of test species such as Stellaria media (L.) Cys., Capsella bursa pastoris (L.) Medic and tobacco (Nicotiana tobacum (Linn.). The alkaloid gramine from barley was found to inhibit the growth of weeds in particularly of Stellaria.

A. Gomez - Pompa (1971) demonstrated that extracts of leaves and fruits of piru (Shinus molle L.) are strongly inhibitory against seed germination and seedling growth of cucumber (Cucumis sativus L.) and wheat.

The fact that extracts of a plant are inhibitory to other plants, however, does not indicate that this plant exerts an allelopathic action against all the other plants. An essential part of allelopathy is the movement of the potential allelopathic agent into the environment. Thus for any suspected allelopathic species leachates of it's leaves, root - exudates, residues and soil previously in contact with it's roots should be tested against potential receptor species from the same locality.

Coutinho and **Hashimoto** (1971) reported that Calea cumeifolia D.C. is a very common under-shrub of Campos - Cerrados vegetation of Brazil, and that pieces of leaves and methanol and ether extracts of leaves of this species

were very inhibitory to seed germination of tomato (Lycopersicum esculentum Mill.) and Mellinus minutiflora Beauv.

Einhelling and **Rasmussen** (1973) found that extracts of leaves of Rumex crispus L. were inhibitory to maize (Gurney's 107-A hybrid), pigweed (Amaranthus retroflexus L.) and to grain sorghum 'Gurney's R 1010) seedlings. Moreover, associated weed species were found to be infrequent in numbers and small in size with in 0.25 m of R. crispus plants in the field.

Sarma (1974a) reported that Digera arvensis forsko., which is a common weed in crops in Rajkot, India, produces toxins which inhibit seed germination and radicle clongation and reduce dry wt. of Bajra Pennisetum typhoides Stapf. and Hubla the latter is an important crop plant in Rajkot.

Solomon, Johnson and **Bhandari** (1981) reported that root extracts of Gomphrena decumbens Facq. were highly allelopathic to the seedling growth of sorghum and sesame.

Colton et al. (1980) reported that Abutilon theophrsti (Medic.) inhibited pod production in soyabeans. The inhibitory action of toxins takes place by stomatal closure and by the lowering of chlorophyll content.

Sharma, Sidana and **Singhvi** (1982) reported the allelo-chemic effect of Peganum harmala Linn. On Pennisetum typhoides Stapf. The aqueous extracts of Peganum harmala Linn. were inhbitory to the growth of P. typhoideus Stapf. The nature of the allelo-chemics changes with the elapse of time.

Schon et al. (1982) in their green house studies demonstrated the allelopathic effects of sunflower cultivar H. annus L. Interstate 894' on the growth and water status of grain sorghum, Sorghum bicolor Moench. Significant growth reductions in sorghum seedlings were found from additions of sun-flower aqueous leaf extracts at Conc. as low as 1 g fresh wt. in 120 ml. nutrient solution.

Bhowmik and **Doll** (1982) investigated the allelo-pathic effects of corn (Zea mays) L. and soyabean (Glycine max) L. Merr. to Weeds. Green fox tail (Setaria virdis) Beauv. and yellow fox-tail S. glauca Beauv, inhibited radicle elongation in soyabeans. Residues of common ragweed Ambrosia artemissifolia L. velvet leaf Abutilon theophrasti Medic. and barn-yard grass Echinochloacrus - galli (L.) Beauv inhibited corn and soyabean growth in the green house. Soyabean yield reduction ranged from 14-19%.

Saleem and **Fawusi** (1983) conducted green house and laboratory experiments to evaluate the allelopathic effects of Euphorbia heterophylla Linn. Cyperus rotundus Linn. and Digitaria sanguinalis Scop. on seed germination and seedling growth of tomato, soko pepper and sorghum. Significant reductions in germination and seedling growth of these plants were observed when grown in pots containing increasing quantities of decomposed weeds.

Stevens et al. (1985) reported the allelopathic effects of root exudates of Bidens pilosa L. on seedling growth of Lactuca sativa L., Phaseolus vulgaris L., Zea mays L., and Sorghum bicolor L. Moench. Their studies involved using a root exudate re-circulating system that allows continuous exposure of the crop plants to allelopathic chemicals. Larger and older B. pilosa plants caused greater inhibition of seedling growth of L. sativa and P. vulgaris than did the smaller younger B. pilosa plants.

Mersie, Wondimagegnehu and **Megh Singh** (1987) demonstrated the allelopathic effect of Parthenium hysterophorus L. on corn Zea mays L., rye-grass (Lolium multiflorum Lam.), wheat (Triticum aestivum L.) velvet leaf (Abutilon theophrasti Medic.)and soyabean Glycine max (L.) Merr. There was a strong co-relation between extract Cone and increasing toxicity to the test-species. The

toxicity of plant part extracts was also Conc. dependent. Toxicity of Parthenium residue to wheat diminished with increasing periods of decomposition. Residue decomposed for four weeks was less toxic than the undecomposed residue.

Mersie et al. (1987) studied the allelopathic activity of Lantana L. on some agronomic crops and weeds. Lantana L. residues affected corn severely and wheat to a lesser extent. The root dry weight of wheat and soyabean was also reduced to a great extent.

Hussain et al. (1988) studied the allelopathic potential of harmal - Peganum harmala L. a weed and waste-land species in Pakistan. Aqueous extracts from shoots significantly reduced the germination and radicle growth of Pennisetum americanum L., Brassica canpestris Linn., Lactuca sativa L. and Trifolium resupinatum L.. Paper chromatography indicated the presence of caffeic acid, ferulic, P. coumaric and P-OH benzoic acid in shoot extracts.

Inam et al. (1989) demonstrated that aqueous extracts from various parts, litter and rain leachates and volatiles from shoots of Cannabis sativa L. significantly retarded either germination, radicle growth, fresh and dry bio-mass or moisture contents of Sorghum bicolor, Moench. Trigonella foenum graeceum Linn., Vigna mungo (L.) Hepper, Trifolium resupinatum L. and Brassica campestris Linn.

2) ALLELOPATHIC EFFECTS OF CROP PLANTS ON WEEDS:

Peters (1968) observed that both thin and dense field stands of kentucky - 31 fescue (Festuca drundinacea Schreb) are often relatively free of weeds. Subsequently investigations with extracts, sand cultures and split root system experiments, demonstrated that toxic materials which were produced by fescue, exuded from the roots and inhibited the growth of Brassica nigra (L.) Koch. and Lotus corniculatus Linn.

Dzubenko and **Petrenko** (1971) reported that root exerections of Lupinus albus L. and of maize inhibited the growth of Chenopodium album L. and Amaranthus retroflexus Linn. and increased their catalase and peroxidase activity.

Neustruyeva and **Dobretsova** (1972) reported that wheat, oats and peas and buck-wheat suppressed growth, accumulation of above-ground bio-mass and leaf surface of Chenopodium album Linn.

Markova (1972) found that oats suppressed the growth of Erysinum cheiranthoides L. owing at least in part to an allelopathic mechanism.

Prutenskaya (1974) reported that wheat (Triticum durum desf.), rye and barley strongly inhibited the weedy Sinapis arvensis L. where as Panicum milliaceum Blanco, stimulates S. arvensis.

3) ALLELOPATHIC EFFECT OF WEEDS ON WEEDS:

Tripathi, Singh and **Rai** in 1981 reported that Eupatorium adenophorum Spreng. which is a common ruderal weed of Meghalaya is highly allelopathic to such herbs as Trifolium repens Linn., Rumex nepalensis Meism., E. riparium Regel., and Paspalum dilatum Poir. The inhibitory effect was co-related with the conc. of the extract. The aqueous extract also caused a significant reduction in leaf number and dry matter production of T. repens.

Datta and **Chakrabarti** (1982) studied the allelopathic potential of Clerodendrum viscosum Vent. in relation to germination and seedling growth of weeds. The activity of decaying plant parts of C. viscosum Vent. and field soils collected beneath Clerodendrum plants were studied on the seed germination and root and hypocotyl growth of 5 weeds, i.e., Abutilon indicum sweet., Amaranthus spinosa L., Cassia sophera L., C. tora L. and Tephrosia hamiltonii. Drumm., Germination was strongly inhibited in A. spinosus, Abutilon indicum Sweet and T. hamiltonii by the underground soil. Decaying roots are most inhibitory. Inhibition occurred in C. sophera L. from the admixture of either plant material and that of T. hamiltonii from leaf/soil misture. As the leaves of Clerodendron form the most consistent source of

the natural chemical retardent these are analyzed and the presence of terpene compound clerodin is suspected in carrying the allelopathic effect.

Rai and **Tripathi** (1982) in their latter work reported allelopathy to be a factor contributing to the dominance of Eupatorium riparum Poir. It caused a reduction in radicle and plumule growth of 4 common weed species i.e. Eupatorium adenophorum Spreng., Galinsoga cilata Blake., G. parviflora Cav. and Trifolium repens L. The extent of inhibition was often co-related with leaf extract conc.

Maryushkina (1983) reported that Bromopsis inermis Leyss. exerts an allelopathic effect on the development and growth of Ambrosia artemissifolia, L. B. inermis cholines stimulate germination growth and development of A. artemissifolia with no shading. But shading promotes a negative allelopathic effect which is especially pronounced in inhibition of root-growth. Sowing of perennial grasses in the loci of A. artemissifolia for it's biological control is suggested.

Al-Saadwi, Ibrahim and **Rice** (1982) reported Polygonum aviculare L. to be highly allelopathic to Cynodon dactylon Linn. Tops and roots of Polygonum, root exudates and leachate of the tops inhibited seed germination and seedling growth of most test-species.

Achhi-reddy, Reddy and **Singh** (1984) studied the allelopathic effects of Lantana camara L. on milk-weed vine Morrenia odorata Lindl. Incorporation of dried Lantana shoot or root material into the soil had no effect on the final % of germination, but caused a significant reduction in milk-weed vine growth over a 30-day test period. Roots were more inhibitory than shoots to milk-weed vine seedlings, 50% died with in 15 days after germination at 1% (wt/wt) Lantana root incorporation. Lantana roots incorporated into the soil produced foliar symptoms such as wilting and dessication, where as Lantana shoots incorporated into the soil produced yellowing of the foliage of milk-weed vine.

Mersie, Wondimagegnehu and **Megh Singh** (1987) reported the allelopathic effect of Parthenium hysterophorus L. extract and residue on some agronomic crops and weeds. Allelopathic effects of entire shoot extracts, plant part extracts and shoot residue of Parthenium on corn (Zea mays L.) rye-grass (Lolium multiflorum Cam.), wheat (Triticum aestivum L.), velvet-leaf (Abutilon theophrasti Medic.) and soyabean (Glycine max. L.) growth were examined. Parthenium shoot contained water-soluble materials that were toxic to root growth of velvet-leaf and wheat. The toxicity of plant

part extracts was also conc. dependent. Residue decomposed for four weeks was less toxic than the undecomposed residue.

Datta and **Ghosh** (1987) reported that 2 species of Chenopodium album Linn. inhibit the germination and seedling growth of certain weeds. Chenopodium ambroisiodes Linn. and C. murale L. decaying leaves, inflorescence and field soils collected beneath Chenopdium plants were examined in terms of the inhibition of seed germination and seedling growth of 5 weeds i.e. Abutilon indicum Sweet., Cassia sophera varpurpurea L., C. tora L., Evolvulus mumularis L. and Tephrosia hamiltonii Drumm. The allelopathic pattern varied in each of the two test species and this depended on the type of test-matter. However, the germination as well as the root and hypocotyl growth of A. indicum Sweet, and E. mumularius L. were more hampered by phyto toxins or inhibitors from Chenopodium than the other weeds. Since the leaf and inflorescence of Chenopodium formed the source of inhibitor the respective plant parts from the two species were chemically analyzed and the presence of three terpenes (P-Cymene, ascaridole and aritzone) from C. ambroisiodes L. and an organic acid (oxalic acid) from C. murale were implied in allelopathic effect.

Ayaz et. al (1989) reported on the allelopathic potential on the allelopathic potential of Adathoda vasica Nees. It is a shrubby component of tropical and sub-tropical vegetation in Pakistan. Aqueous extracts, rain leachates, litter from the shoots and soil underneath it invariably reduced germination, early growth, bio-mass, moisture and chlorophyll content of some crops and weed species. Chromotographic analysis revealed the presence of caffeic, ferulic, vanillic, p - coumaric, p - OH - benzoic and tannic acids in aqueous extracts. The phyto-toxicity was related to the test - species used, plant part assayed and the parameters measured. It was suggested that the preclusion of the associated species and the dominance of A. vasica is primarily due to allelopathy.

Smith and Albert (1989) suggested that Helenium amarum (Rafin) or bitter sneezeweed as it is commonly called is highly allelopathic to some weeds such as alfa-alfa and Italian rye-grass. It reduces their growth by as much as 50% at conc. of 0.5% Leaf extracts were more phyto-toxic than either stem or root extracts and seedling growth was reduced more than seed germination. Bitter sneezeweed tissue mixed in potting soil at concentrations as low as 0.3% w/w reduced alfa-alfa seedling numbers by 43% plant

ht., by 26% and foliage dry matter production by 54% compared to plants cultured in soil without bitter sneezeweed leaf tissue. The potential conc. of bitter sneezeweed leaf material in soil in the pasture eco-system was determined to be 0.5% w/w.

Srikanth and **Pushpalatha** (1991) reported the status of biological control of Parthenium hysterophorus L. in India. Parthenium is a native of tropical America, this weed came to India with imported food grains and now it has invaded wide agricultural and waste lands.

Parthenium hysterophorus L. can be controlled by Cassia uniflora Mill. (Leguminosae). toxic leachates of C. uniflora Mill (Leguminosae) amd auto-toxic principles of the weed are inhibitory to Parthenium. Due to the failure of other methods allelopathy can prove to be very promising for biological control of Parthenium.

ALLELO CHEMICALS: THEIR MECHANISM OF ACTION

Allelo chemicals or secondary plant products are shown to have potential of suppressing higher plants' growth, regardless of whether or not, these are physiologically active in any vascular or non-vascular plant. (Muller and Chou 1972). Among various groups of phytotoxins, plant phenolics and terpenes have been reported relatively more.

Workers have used these plant products from higher plants or from lower organisms against higher plants as herbicides. Plant phenolics comprising phenolic acids, coumarins, flavonoids, quinones and tanins have been implicated in allelopathy (Whit taker and Fenny, 1971; Rice 1974). Their benzoic or cinnamic acid representatives are often shown to occur in plants in sufficient amounts as esters or glycosides. Their escape from the plant is reported through leachation. Such allelo-chemics are supposed to alter respiration rates in the target plants through inhibition or stimulation of oxygen uptake (Lodhi and Nickel 1973; Demos et al., 1975). They also alter protein synthesis and membrane permeability.

Apart from phenolic acids, coumarins (reported to be prevalent in grasses, legumes and citrus plants (Robinson, 1980) possess relatively more potential as regards their allelopathic effects (Duke et al., 1984). Coumarins inhibit both oxidative and photo-phosphory-lation (Yakuchkina and Starikova, 1977). Flavonoids normally found as sugar conjugates in plants have also been shown to exhibit allelo-pathic effects (Carballirea, 1980).

Tanins (the water soluble poly-phenolic acids) as well as the condensed tanins have been reported to inhibit growth and germination of a few plants. These have been shown as antagonists of gibberellic acid induced growth (Green and Corcoran, 1975). Further, alkaloids (Chou and Waller, 1980a and b) quinones (Putnam, 1983, Rice, 1984) and terpenoids (Duke at el, 1987 Stevens and Merill, 1985, Kumari, 1988 and Kushal-bala, 1987) have also been included in the list of phyto-toxins that have allelopathic impact on plants.

USE OF NATURAL PRODUCTS IN CRUDE FORMS AND IDENTIFICATION OF POTENTIAL PHYTO-TOXINS FROM HIGHER PLANTS:

Green plants produce hundreds of thousands of compounds that are not involved in the primary metabolism of the plants, hence the term "secondary metabolites" has been

used for such compounds. However, the functions of these compounds remains obscure. They are thought to be involved in interactions of plants with other organisms like pathogens, insects, nematodes and other plants. The compounds (phyto-toxins) involved in inter-specific chemical interactions with higher plants are often phyto-toxic or herbicidal to other species or to the species producing them (auto-toxicity). In nature these are released in crude forms, through volatilization, leaching, degradation or as root exudates and affect other plants growing in their vicinity.

MECHANISM OF ACTION OF ALLELO CHEMICALS

The elucidation of mechanism of action of allelo-chemicals by which they alter the growth and development of the plants is a difficult and on-going question. The reason for this is, limited amount of work available. It must be emphasized that much more work on this field is needed, to get a clear insight into the precise physiological perturbations. Based on the available data, the modes of action of allelo-chemicals could be studied under the following heads:

REGULATION OF GROWTH:

a) Cell-division, elongation and ultra-structure:

Several efforts to characterize the allelo-chemical action support the inference that cell division and elongation are adversely affected by the allelo - chemicals. Saturated solution of coumarin blocks all the stages of mitosis in Allium cepa L. (Cornman, 1946). Terpenes having volatilized from the macerated leaves of Salvia leucophylla Greene,, completely check mitosis (Muller, 1965). These terpenes, besides checking mitosis also prevent the cell elongation of roots and hypocotyls. Later Lorber and Muller (1976) reported that exposure of cucumber (Cucumis sativus L.) roots to volatile terpenes from Salvia leucophylla Greene, cause accumulation of globules in the cytoplasm of root tips of the former. Umbelliferone has been reported to decrease the rate of cell elongation in the Cucumis sativus L. roots and increase radial expansion of it's cells (Jankay and Muller, 1976). Seedlings of C. sativus died because of disruption of meristems. Leaf expansion of C. sativus L. has been checked by phenolic acid treatment (Blum et. al., 1985). Artemisinin, a sesquiterpene lactone adversely affects the mitosis in Lactuca sativa L. (Duke et al., 1984).

b) Organic Synthesis:

Bio synthesis of major plant constituents or the distribution of carbon in cellular pools is modified by various allelo - chemicals, particularly the phenolic. Uptake and incorporation of phenylalanine 14C by yeast (Saccharomyces cerebrisae) cells was inhibited by a group of Cinnamic, benzoic acids and coumarins (Van sumere et. al., 1972). Further studies on these showed that these events were inhibited in Lactuca sativa L. seeds and Hordeum vulgare L. embryos. There are likewise several reports of protein inhibition indicating the effect on growth. Kolesnichenko and Aleikina (1976) reported the low rate of protien bio-synthesis in the roots of Quercus robur L. growing close to the roots of Fraxinus excelsoir L. than in those growing in the vicinity of Quercus. Cinnamic and ferulic acids (0.05 mm) significantly inhibit protien synthesis in Lactuca sativa L. seedlings when added from the beginning of germination period or when added for a short period compared to the seedling, germination under controlled conditions (Cameron and Julian, 1980). Likewise, inhibition of protien content in Vigna unguiculata Var. RML - 1 has also been reported by Kanwaljit (1986) in response to the Eucalyptus allelo - chemics.

c) Inter-actions with Hormones:

The interaction of allelo-chemicals with hormones has been an intriguing question. Stenlid (1968), reported that naringenin 2,4,4-tri-hydroxy Chalcone and phloridzin in combination with some related flavonoid glycosides are strong stimulators of IAA oxidase. Wurzberger and leshem (1969) reported the inhibition of gibberellin, but not of IAA induced growth by some germinator inhibitior in the glumes and hull of Aegilops kotchyi Boiss.

Hypocotyl growth was seen to have been inhibited when induced by GA, but not by IAA by six chemically defined tanins in Cucumis sativus L. seedlings (Geissman and Phinney, 1972). The gibberellin induced growth of dwarf pea (Pisum sativum L.) gets inhibited by many chemically defined tanins (Corcoran et. al., 1972; Greene and Corcoran, 1975). Contrary to reduced growth, by binding gibberllic acids, there are instances where enhanced growth has been seen by binding abscissic acid (ABA). Ray et. al., (1980) reported that many phenolics release ABA inhibitor . Coumarin, ferulic, gallic, tannic and cinnamic acids remove ABA inhibition of hypocotyl growth in Amaranthus caudatus Linn.

d) Effect on Enzymes:

Allelo-chemicals alter either the synthesis or function of many enzymes. Jankay and Muller (1976) reported

that unbelliferone caused a swelling response in Cucumis sativus L. roots and resulted in increased peroxidase levels. Jain and Srivastava (1981) reported that nitrate reductase activity in Zea mays L. gets increased by 10 µ M salicylic acid and was inhibited above 1000 µµ M. The effects were thought to be on enzyme synthesis.

1,3,7 - trimethyl xanthine, an allelo-chemic from the seeds of Coffea arabica L. caused a marked decrease in amylase activity in the seeds of Amaranthus spinosus L. when treated (Rizvi et. al., 1987).

2) **RESPIRATORY METABOLISM:**

Patrick (1955) found that water extract of soil in which decomposing leaves of Prunus persica were present, inhibit respiration of P. persica itself. Later, it was found that water extracts of soil in which decomposing residues of several crops were present inhibit respiration in excised Nicotiana tobacum L. roots. (Patrick and Koch, 1958; Patrick et al., 1964).

Two volatile terpenes which emante from the leaves of Salvia leucophylla Greene., markedly reduced O_2 uptake by suspensions of mitochondria from Avena fatua L. and Cucumis sativus L. (Muller et. al., 1965).

Inhibition of respiratory metabolism may represent an important mechanism of action in the hypocotyls of Phaseolus aureus Roxb. Seedlings in response to ten phenolics (Demos et. al., 1975). Allelo-chemics produced by Pondorina morum were shown to inhibit electron transport in Solanum tuberosum L. mitochondria (Patterson et al., 1979). Andreo and Orellano (1984) reported that Indole - alkoloid-gramine inhibits photo-phosphrylation, pi-ATP exchange reactions and proton gradient while enhancing electron transport in Spinacea oleracea L. thylakoids.

3) PHOTO-SYNTHESIS AND RELATED PROCESSES:

a) Photo-Synthesis:

The increase in dry matter of higher plants is linked to carbon fixation, so any loss in efficiency of photo-synthesis might be deterimental to growth. Einhelling et. al., (1970) reported that scopoletin markedly inhibits the photo-synthesic rate of intact plants of Helianthus annus L., Nicotiana tobacum L. and Amaranthus retroflexus L.

Photo-synthesis could be altered by different mechanisms. It could be due to direct effects on the chloroplasts or indirectly by effecting stomatal closure. Arntzen et. al., (1974) found that Kaempferol - a flavonol

inhibits coupled electron transport and both photo-phosphorylations (Cyclic as well as non-cyclic). In contrast, dihydrochalcone gluoside and phlorizin inhibit the activity of chloroplast membrane.

b) Stomatal Response:

Scopoletin and Chlorogenic acids in concentrations 10^{-3}M and 5 x 10^{-4}M exhibit stomatal closure in Helianthus annus L. and Nicotiana tobacum L. where as the conc. of 10^{-4}M stimulates the opening of stomata (Einhelling and Kuan, 1971).

Victorin - a type of marasmin, reduces stomatal aperture and decreases the rate of transpiration in treated Avena sativa L. plants, whereas fusicoccin, which is another type of marasmin shows opposite results (Turner, 1972). The introduction of water extracts from velvet leaf (Abutilon theophrosti. Medic), Kochia (Kochia scoparia L.) Schrad., Jerusalem artichoke (MI Helianthus tuberosum L.) and Cocklebur (Xanthium pensylvanicum Wallr.) into the nutrient solution for growing grain sorghum (Sorghum Sp.) and soyabeans (Glycine max L.) (Me rr.) caused stomatal closure (Colton and Einhellig, 1980; Einhelling and Schon, 1982.)

c) Chlorophyll Content:

A chlorotic appearance has occasionally been reported in allelopathic interference. Einhelling and Rasmussen (1979) found that treatment with ferulic, p-coumaric and vanillic acids result in loss of weight and less chlorophyll content in Glycine max (L.) Merr. plants, Chlorophyll loss could contribute to lower rate of photosynthesis. Similarly Kumari et al., (1985) reported the loss of chlorophyll in the leaves of Brassica campestris L. and Parthenium hysterophorus L. in response to the allelochemics of P. hysterophorus L. itself.

NUTRIENT UPTAKE AND ASSOCIATED PROCESSES

Most of the allelo-chemics affect the emergence of roots. Since, roots are involved in mineral uptake, it has therefore been contemplated that allelo-chemics affect mineral uptake of the test-species. On the basis of several experiments on Hordeum vulgare L., Glass (1973-74) reported that all the phenolic acids tested inhibit the uptake of labelled phosphate (P^{32}). The degree of inhibition co-related well with the lipid solubility of the compounds. The membrane potentials of H. vulgare L. root cells were rapidly depolarized by several benzoic and cinnamic acid derivatives and there was a strong co-relation between deplorization values for benzoic acids and lipid solubilities (Glass, 1973; 74 a,b; Glass and Dunlop, 1974). Vanillic acid has been seen to reduce mito-chondrial calcium ion uptake (Demos et al. 1975).

The alteration of mineral content in plants has been studied greatly. Since, mineral uptake requires energy, this may lead to an effect on oxidative phosphorylation, or inhibition of plasma membrane bound ATP. Balke (1977) surveyed various phenolic acids for their effects on potassium ion absorption by Avena sativa L. roots and ATPase activity of plasma membrane vesicles isolated from it.

Juglone was found to be most inhibitory Balke and Hodges (1977) supporting this found that synthetic diphenolic compound, diethylstilbestrol (DES) markedly inhibit potassium and chloride ion absorption by excised oat (A. sativa L.) roots. ATP-ase bound to plasma membrane was also inhibited.

Kobza and Einhellig (1987) reported that ferulic acid reduces the concentrations of phosphorus and potassium ions in both root and shoot of Sorghum bicolor (L.) Moench, thereby affecting the balance of other nutrients.

EFFECTS ON WATER RELATIONSHIPS

Since, mineral uptake and membrane functions are altered, it is bound to affect the plant-water balance. Leaf water potential was seen to have been lowered in Glycine max L. Merr. and Sorghum bicolor (L.) Moench. by the treatment of p-coumaric and ferulic acids (Einhellig, et al., 1985). Similarly Patterson (1981) reported that depressed growth of Glycine max (L.) Merr. treated with 1000μ M caffeic, ferulic and gallic acid was due to the reduction in water potentials.

Likewise Colton and Einhelling (1980) Schon and Einhelling (1982), Einhelling et al. (1985) have shown

water stress as an action of Albutilon theoprosti. Medic, Kochia scoporia (L.) Schrad., Helianthus tuberosus L. and Xanthium penslyvanicum Wallr.

EXPLOITATION OF ALLELOPATHY FOR WEED MANAGEMENT

Weeds are integral components of agro-eco-systems. Due to their evident impact on crop yields, weeds have traditionally been considered unwanted plants. Consequently agriculturists have concentrated their research on weed/crop interference and weed reproduction, with relatively less focus on an analysis of the ecological mechanism involved. Generalizations about crop yield losses due to weeds have justified the promotion of season - long, weed-free crop systems that rely on the use of costly chemical herbicides (Aldrich, 1984). The excessive use of chemicals or herbicides/weedicides seems impracticable. It is so for two counts. Firstly, the cost factor and secondly, the chemicals used are not degraded easily in terms of time and nature.

For the last over two decades, there has been an increasing concern towards the use of biological renewable agents towards weed management. There are several species known that have successfully managed through the introduction of natural predators. Pesticides from plant sources are more systemic and easily bio-degradeable than synthetic pesticides (Rizvi et. al., 1984). Same is true about

herbicides. It is argued by some that every plant release some amount of one or the other chemical to adjust itself in the abiotic component and the biotic component of its eco-system. Depending upon the amount, nature and thereby, the action of the release from the plants, some plants that adversely affect the growth of neighbouring or subsequent plants or biota are regarded as allelopathic. The fact that plants produce secondary products, in majority the allelochemics that get released in the environment through volatilization, root exudation, aerial leaching and decomposition of plant residues (Rice, 1984) and affect adversely the growth of other plants is tempting the mankind for exploiting it towards weed control and management.

PLAN OF WORK

Allelopathy has been recently developed as a branch of science with connections in Agriculture, Botany, Bio-Chemistry, Chemistry, Ecology, Horticulture. Social-forestry etc.

Allelopathic studies involve the extraction of the various allelo-chemicals from the plant and from the soil where it is growing. The identification and characterization of these chemicals is a complex and long process. The test-plants are treated with various concentrations of these chemicals to note the effects of these allelo-chemicals on the plants. It involves a series of complex bio-chemical techniques as chlorophyll estimation, carbohydrate content, protein estimation, R.N.A. estimation etc.

The following main stages shall be taken into consideration:

1) Choice of group, taxonomic survey and sound sampling.
2) Bio-chemical techniques.
3) Statistical analysis.

TAXONOMIC SURVEY

This stage covers the choice of taxonomic group to be investigated. For this purpose families labiatae, orobanchaceae and Acanthaceae have been considered.

BIO-CHEMICAL TECHNIQUES

1. Extraction of leachable allelo-chemics and organic fractions and extraction of the main allelo-chemic from the plant.

2. Extraction of soil chemics.

3. Germination trials for each treatment.

4. Estimation of chlorophyll content.

5. Cell survival test.

6. Water content, estimation.

7. Carbohydrate estimation.

8. Estimation of the activities of enzymes

STATISTICAL ANALYSIS

The last and final stage of the study involves, compilation of the data, its analysis and interpretation for results.

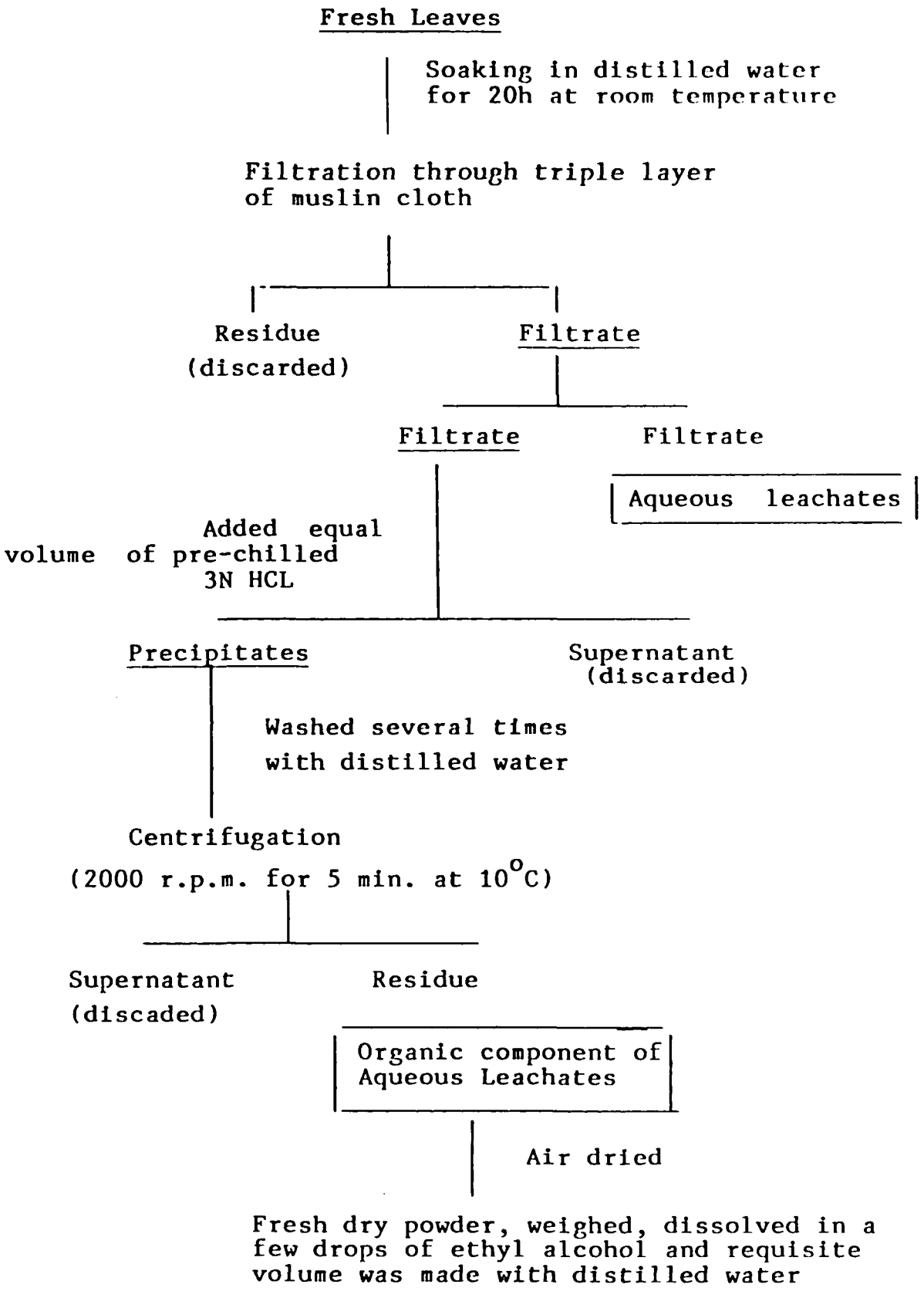
BIO-CHEMICAL TECHNIQUES

Extraction of Leachable Allelo-Chemics (Protocol I)

Based on the methods devised by Kumari et. al (1985), freshly collected surface cleaned and healthy leaves of the plant are cut roughly into pieces and their dry weights per unit fresh weights are determined by desicating the tissue in the oven.

The weighed amount of fresh leaf pieces of the plant are soaked in requisite amount of pure water (resistivity more than 18.5 mega ohms cm and conductivity less than 0.05 u Simons cm at $25^{o}C$) for 29 hours. It is filtered completely through muslin cloth and the requisite conc., is made with water. One part of this filtrate which as the 'Aqueous leachate' is used as such, while the other part is chilled and subjected to acid hydrolysis using pre-chilled 3N HCl (Trim 1953). The precipitates so formed are recovered through centrifugation (2000 rpm). These are washed 5 to 6 times with pure water. Every time, the recovery is made through centrifugation. For experimental purpose requisite amount of precipitate is dissolved in a few drops of ethyl alcohol and the final amount is made with pure water. A drop of 'tween 20' is added to serve as

surfactant. This is referred to as 'A glycone' or A glyconic or organic component of Aqueous leachates.



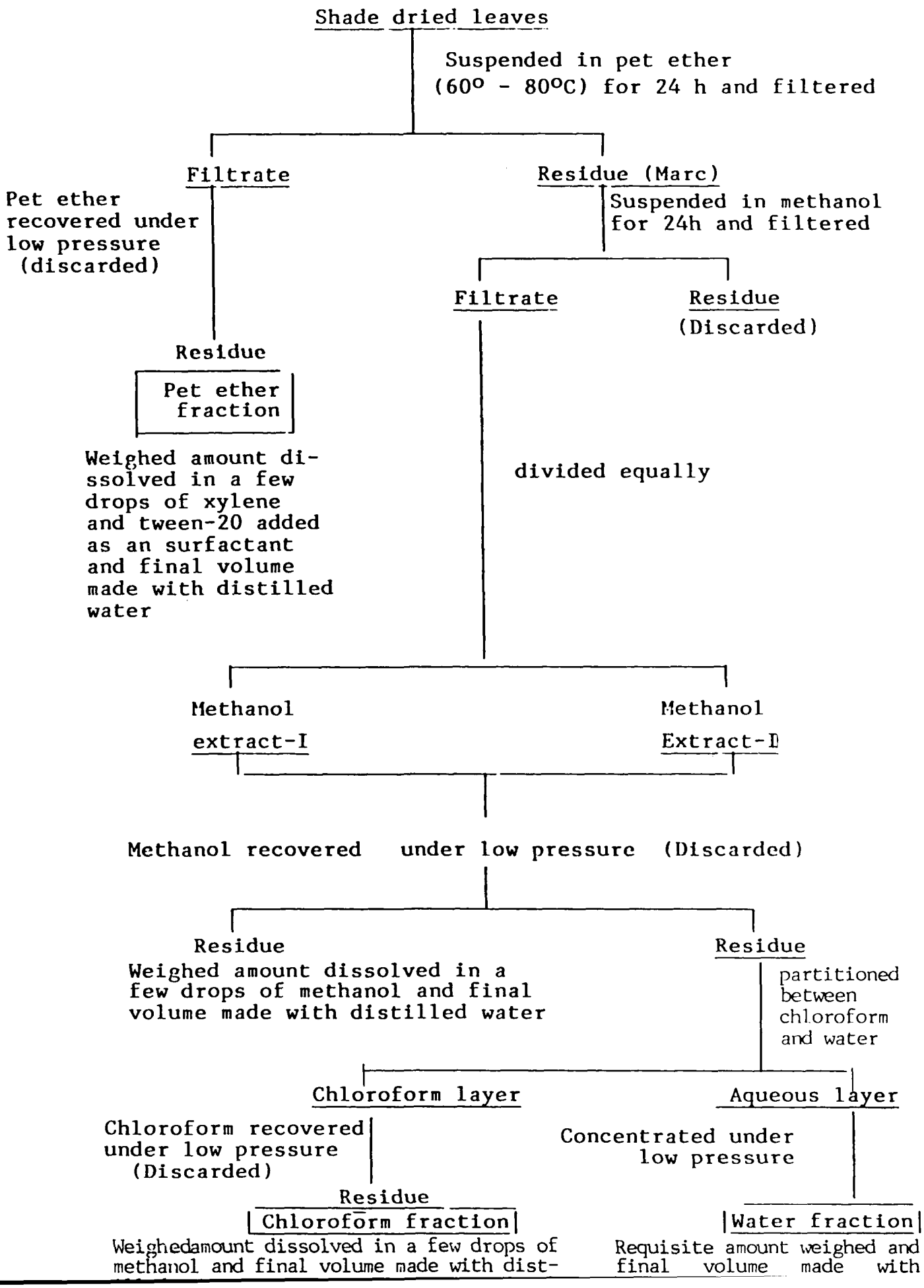
Protocol I: Extraction of aqueous leachates and organic component of aqueous leachates (After Kumari et al., 1985).

Extraction of Organic Fractions (Protocol : II)

Freshly collected, surface leaned, healthy leaves of the requisite plant are dried under shade and powdered. The powder is immersed in petroleum ether (60-80^{o}C) for 20 hours. The liquid is seperated from the residue (Marc), through mild centrifugation (500 rpm for 2 min.). From the liquid portion the solvent (petroleum ether) is recovered on a hot water bath. Requisite amount of the residue so obtained is weighed and a few drops of xylene, apart from a drop of tween-20 (to act as surfactant) is added to it. The final volume is made with pure water and this is termed as the petroleum ether fraction.

The marc (residue from the Petroleum ether suspension) is suspended in methanol for 20 hours and filtered. From one half of the filtrate, methanol is recovered on a hot water bath. The residue, so obtained is dissolved in a drop of methanol and the final volume is made with water. It is called Methanol Fraction (MF). From another half of methanol filtrate, the solvent is removed and the residue is partitioned between chloroform and water (1:1, v/v). The two layers so formed are separated in a separating funnel. The chloroform is recovered over a hot water bath. To the

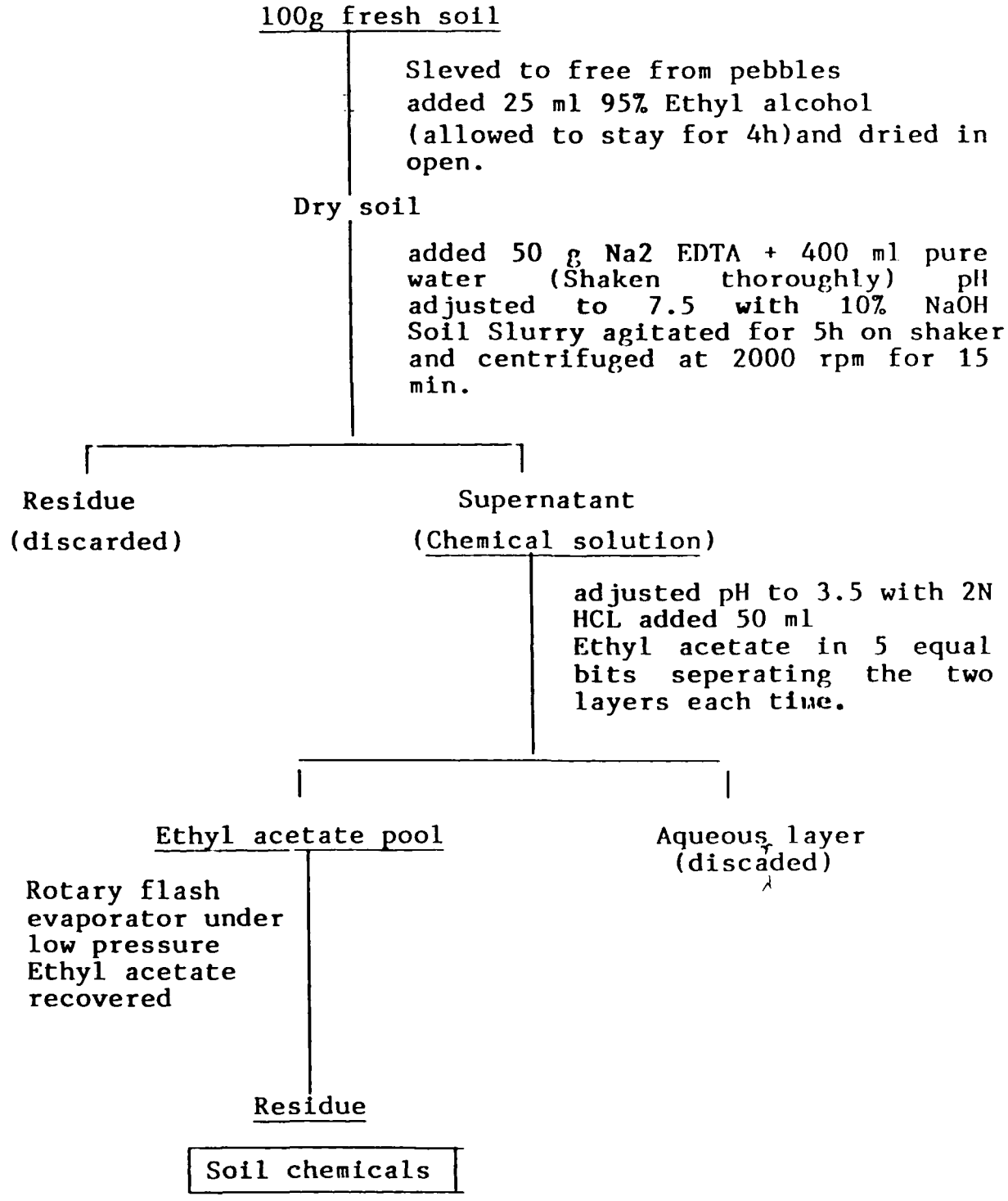
requisite amount of the residue a few drops of methanol are added and the final volume is made with pure water. This has been termed Chloroform Fraction (CF). The water from the aqueous layer after separating the chloroform fraction is dried under low pressure on a rotary flash evaporator. The solution made with water is termed as the Water Fraction (WF).


Shade dried leaves
Suspended in pet ether (60° - 80°C) for 24 h and filtered
Filtrate
Residue (Marc)
Pet ether recovered under low pressure (discarded)
Suspended in methanol for 24h and filtered
Filtrate
Residue
(Discarded)
Residue
Pet ether fraction
Weighed amount dissolved in a few drops of xylene and tween-20 added as an surfactant and final volume made with distilled water
divided equally
Methanol extract-I
Methanol Extract-II
Methanol recovered under low pressure (Discarded)
Residue
Weighed amount dissolved in a few drops of methanol and final volume made with distilled water
Residue
partitioned between chloroform and water
Chloroform layer
Aqueous layer
Chloroform recovered under low pressure (Discarded)
Concentrated under low pressure
Residue
Chloroform fraction
Water fraction
Weighedamount dissolved in a few drops of methanol and final volume made with dist-
Requisite amount weighed and final volume made with

Extraction of Soil Chemics
(Protocol : III)

Soil samples collected from the area inhabited by the selected plants are immediately sieved to remove pebbles and debris, etc. The soil chemics from it are extracted following the method of Kaminsky and Muller (1977) with slight modifications. To 100 g freshly collected soil is added 25 ml of 95% ethyl alcohol, in order to suppress microbial activity and thereby, preventing the alteration of the components. Five hours later 25 g of sodium salt of EDTA and 200 ml of pure water is added. The mixture is shaken carefully for several minutes to allow EDTA to dissolve completely after which the pH of the suspension is adjusted to 7.5 with 10% NaOH. It is subjected to continuous slow motion shaking for 5 hours. The contents are centrifuged for 15 min at 2000 rpm and the supernatant is decanted through glass wool. The pH of the supernatant is adjusted to 3.5 with 2N HCl. The chemics are extracted in 5 volumes of ethyl acetate, 50 ml, each time.

The ethyl acetate is pooled and the solvent is recovered at low temperature. The residue is termed soil chemics.



Requisite amount dissolved
in a few drops of ethyl alcohol
and final volume made with pure water

Protocol III: SCHEME OF EXTRACTION OF SOIL CHEMICS (After Kaminsky and Muller, 1977 with slight modifications)

GERMINATION TRIALS

Seed germination trial is performed following International seed testing association, 1976 (ISTA 1976) rules. Uniform, viable seeds of the plant under test are collected from weed thickets. These are surface cleaned and subjected to germination trial in response to the treatments. For each treatment 400 seeds of the requisite type are taken and these are soaked in respective treatment solutions at room temperature for 18 hours. Treatment with pure water to a set of four hundred seeds serves as control. The soaked seeds of each treatment are placed in petri-dishes in such a way that each houses 100 seeds. For this purpose each Petri-dish is lined with a thin absorbent cotton pad with Whatman No.40 filter paper. The bed is moistened with respective treatment solution properly. The seeds are arranged in concentric rings maintaining a proper inter seed space.

The set up is maintained in a seed germinator at 30 $\pm$ 2^{o}C temperature, relative humidity of 30 $\pm$ 1% and continuous light of approximately 4000 lux for 24 hours daily, observation are made daily and the seed vigour is calculated.

Treatment to the Plants: The Plants of the selected species are raised in experimental plots, they are given a fine mist of aerial spray (20 ml/plant) of requisite concentration of requisite treatment solutions for 3 consecutive days. Spray of pure water serves as control. On the day, following the last spray, the leaves are plucked, surface cleaned with a brush and utilized for estimations.

ESTIMATIONS

Chlorophyll Contents: The total chlorophyll content from the leaves of treated or control plants is extracted in Dimethyl sulphoxide (DMSO) following the method of Hiscox and Israelstam (1979) with improvements as suggested by Daizy and Kohli (1991). Finely cut uniform discs (100 mg fresh weight) are made from fully expanded leaves of the plant.

Dry weight equivalents of each of the test samples are determined by keeping 100 mg fresh wt. disc in an oven.

The weighed material (100 mg fresh weight leaf disc) is suspended in 10 ml of Dimethyl sulphoxide (DMSO), incubated at 65^{o}C for one hour (the period of incubation is found sufficient for the complete extraction of chlorophyll). The DMSO is recovered by thorough decantation. The final volume is corrected to 10 ml with fresh DMSO. The extinction of chlorophyll thus recovered in DMSO is measured at dual wave-lengths of 645 and 663 nm on spectronic 1201 Spectrophotometer against DMSO as blank. The total amount of chlorophyll is calculated.

Cell Survival Test: The cell survival values are calculated following the method of Steponkus and Lanphear

(1967) with slight modifications as suggested by Daizy (1990). Uniform discs of 100 mg fresh weight are made from freshly harvested fully expanded leaves of plants (control as well as treated). Dry weight equivalents of each of the samples is found by keeping 100 mg discs of the fresh leaves in an oven at 80°C for 24 hours.

To 100 mg (fresh wt.) discs is added 1.5 ml of freshly prepared 0.6% (W/V) 2,3,5-triphenyl tetrazolin chloride (TTC) in 0.1 M phosphate buffer (pH 7.4). A wetting agent Tween-20 is added to the solution in the concentration of 0.05% (V/V). It is then subjected to incubation at 26°C for 15 hours in the dark. Each test-tube containing the incubated material is covered with a bi-layer of muslin cloth from the rim and inverted to drain off the TTC Tween-20 solution. A gentle washing to the leaf discs is given by immersing the muslin cloth covered rim in pure water. The water insoluble formazan is extracted from the leaf discs twice with 2.5 ml of 95% ethyl alcohol for 10 min. over a water bath at 60°C. The extracted formazan is pooled and the final volume is made to 5 ml with 95% alcohol. The pooled extract is cooled at room temperature and the extinction is read at 530 nm on spectronic 1201 Spectrophotometer. The values are compared further and calculated as per dry weight of control. The cell survival values are expressed as percentage with respect to control.

WATER CONTENT

The content of water is measured by the apparatus originally devised by Dean and Stark (British Pharmacopia, 1980, Vol. II, P. Agg.) following the method given by Trease and Evans (1983). The method is considered better over fresh and dry weight method because of the following reasons against the latter.

a) Volatile component, if any, in the material gets removed on drying in the oven adding to the loss of weight.

b) Possibility of gain of weight due to rehydration of dried sample from humid air during the period between removal of sample from the oven and bringing to the room temperature for weighing.

For determining the water content, water saturated Xylene and a few pieces of porous pot are added to the weighed sample in a round bottomed flask and distilled. Due to considerable partial pressure, the water gets co-distilled with the solvent and condenses forming an immiscible layer with the solvent. The amount of water,

thus collected is measured directly in the apparatus. The per cent water content is measured and calculated as per gram dry weight over control.

CARBOHYDRATE CONTENT

The method of Loweus (1952) is followed for this purpose.

EXTRACTION:

a) **Water Soluble Carbohydrate:** To 5 mg of dry powdered - material is added 5 ml of pure water. It is kept in boiling water bath for 20 minutes. After centrifugation, the supernatant is stored as the water soluble fraction (WSF).

b) **Acid Soluble Carbohydrate:** To the residue left as above is added 5 ml of 6N HCl. This is kept in a boiling water bath for 5 min. and centrifugated. The supernatant is used as Acid Souble Fraction (ASF).

ESTIMATION

To 1 ml solution (each of WSF or ASF) is added 4 ml of Anthrone Reagent (0.2% Anthrone dissolved in conc. H_2SO_4). The tubes are kept in boiling water bath for 10 min. The concentration of the carbohydrates from brownish yellow to green colour is read at 620 nm by pressing concentration key in the dual beam spectronic 1201 Spectrophotometer against a known concentration of glucose as standard. The carbohydrate content is expressed as mg/g dry weight of material.

ESTIMATION OF THE ACTIVITIES OF ENZYMES

The thoroughly washed tissue is chilled in a freezer. It is homogenized in a pre-chilled glass pestle and mortar, using a pinch of acid washed (pH 7.0) sand and a little of the extraction buffer (1.19 g/100 ml Na_2HPO_4 and 1.04 g/100 ml NaH_2PO_4 mixed in the ratio of 1:1 (V/V), pH 7.0). The homogenate is centrifugated at 8000 xg for 7 minutes, followed by recentrifugation at 17,000 x g for 11 minutes and finally at 27,000 x g for 5 minutes. The centrifugations are done at 4^oC of the tube temperature. The clear supernatant is collected. The concentrations of

the various extracts are equalized with respect to protein content with extraction buffer.

α - AMYLASE (E.C. 3.2.1.1.): It was estimated by the method of Muentz (1977).

Substrate Solution: Boiled 150 mg of soluble starch, 600 mg of K_2 HPO_4 and 20 mg of an-hydrous calcium chloride in 100 ml of pure water for 1 minute is cooled and filtered.

Iodine Solution: 25.4 mg Iodine is dissolved in 0.4 g KI in 100 ml of pure water.

Estimation:

To 1 ml of the clear substrate is added 1 ml of the enzyme extract. It is incubated for half an hour at 30°C and then 1 ml of 0.1 M EDTA is added to it. 0.2 ml of the reaction mixture is added to 3 ml of iodine solution. The concentration of the left over starch is measured directly using a known concentration of starch at 620 nm on dual beam spectronic 1201 Spectrophotometer. Control is prepared without enzyme extract. The specific activity is measured in terms of starch used and expressed as µg/min/mg protein.

β - AMYLASE (E.C. 3.2.1.1): The activity is measured by the method of Bernfeld (1951), modified by Dure (1960).

Dinitro-salicylic Reagent: To 50 ml of pure water is added 2.5 g of Dinitro salicylic acid (DNSA). After dissolving it completely, 4 g of NaOH is added. To it is added 75 g of sodium potassium tartarate and the final volume is made to 250 ml with pure water.

Estimation:

To 0.7 ml of the substrate solution, is added 0.1 ml of 0.1 M EDTA and 1 ml of enzyme extract. The reaction mixture is incubated at 30°C for 30 min. After this, the reaction is stopped by adding 1 ml of DNSA. The tubes are kept in boiling water bath for 20 min. These are removed and cooled to room temperature and 3 ml of pure water is added in each. The extinction value is measured at 560 nm against a known concentration of maltose.

The specific activity is measured in terms of maltose units released per gram protein and it is expressed as μg/min/mg protein.

STATISTICAL ANALYSIS

Sampling for experiments involving germination parameters (Per cent of germination, mean radicle and plumule length); chlorophyll content, cell survival, water content, carbohydrate content and activities of enzymes are made randomly in control and the treated cases. For each case a minimum of three replicates are maintained. The experiments are performed at least twice. The first is considered as preliminary in order to ascertain the treatment concentration and the other parameters, whereas the second based on the preliminary data is considered final for reporting the observations.

The final values are subjected to analysis of variance (ANOVA) so as to calculate the F value. The calculations are based on computerized programme as given by Lee and Lee (1982). The differences among several means over that of control stand significant if the calculated value of F exceeds that of tabulated value at 5% level of significance. Since the significance of difference among the mean values of treatments cannot be ascertained by this test, multiple range test as devised by Duncan (1955) is applied at 5% level of significance.

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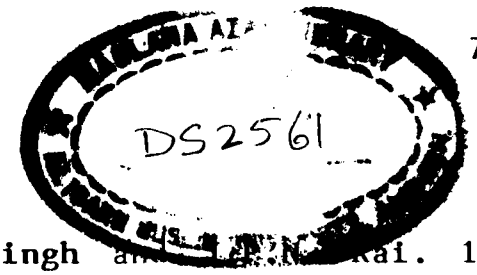

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